Importance of Serum Prolactin Determination in Metastatic Breast Cancer Patients

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Aim. To correlate prolactin concentrations in the sera of patients with metastatic breast cancer with time interval to appearance of metastases, their location, and size.

Method. The prospective study included 46 female patients with histologically confirmed diagnosis of breast cancer. The patients were recruited from the Health Center outpatient clinic and University Hospital Center day-care hospital in Banja Luka, Bosnia and Herzegovina, from January to August 1988. The follow up lasted 5 years. Serum concentrations of prolactin were measured in all patients before (baseline levels) and after the therapy at regular time intervals during the observation period. Their prolactin concentrations were compared with prolactin concentrations in 40 healthy women and 33 female patients with other types of cancer, who served as control groups. Time interval to metastases development, their size, and location were determined in breast cancer patients and compared with those in patients with other types of cancer.

Results. The baseline serum concentrations of prolactin were higher in breast cancer patients than in healthy women (610 vs 442 mU/L; p=0.04; Mann-Whitney test), and in patients with other locations of cancers (662 vs 481 mU/L, respectively; p=0.02; Mann Whitney test). Metastases developed in all hyperprolactinemic patients, whereas a third of normoprolactinemic were free of metastases. The average time interval before the occurrence of metastases in patients with very high serum concentrations of prolactin was significantly shorter than that in patients with very low prolactin concentrations (54.3 vs 6.1 months; p<0.001; Mann Whitney test). In hyperprolactinemic patients with metastatic breast cancer, there was a significant correlation between the serum concentration of prolactin before treatment and the time to metastases (r = -0.47; p = 0.03) and the size of metastases (r = 0.64; p = 0.001).

Conclusion. Hyperprolactinemia could be an indicator of disease progression and poor prognosis in patients with metastatic breast cancer.

Key words: breast neoplasms; hyperprolactinemia; neoplasm metastasis; prognosis; prolactin

Accumulated data about the role of prolactin in human breast cancer are controversial. Most studies suggest that this hormone plays an important role in breast cancer initiation and development in rodents (1,2) and, at least partly, in humans. There is also firm evidence of a direct stimulatory effect of prolactin on mammary epithelial cells (3,4), and breast cancer cells in culture (5). Several studies have reported that prolactin is produced by both normal and malignant mammary cells and thus may be an autocrine/paracrine factor for this tissue (6,7). There are also some epidemiological studies showing significant increase in serum prolactin concentrations in certain subpopulations of breast cancer patients (8-10), and in women at risk of developing familial breast cancer (11), whereas other studies did not confirm these findings (12). This hormone may play a role in two separate processes — carcinogenesis and maintenance (probably via the stimulation of growth) of already established populations of breast cancer cells. Since the clinical data on the concentrations of circulating hormone in breast cancer patients are controversial (12-14), further investigations into the role of prolactin in breast cancer are justified.

Our aim was to evaluate diagnostic usefulness of prolactin in breast cancer patients and the relationship between its circulating concentration and the essential metastatic parameters, such as time to metastases, their location, and size.

Patients and Methods

Patients

Patients were recruited from the Health Center outpatient clinic and University Hospital Center day-care hospital in Banja Luka, Bosnia and Herzegovina, from January to August 1988. The inclusion criteria for female patients with breast cancer were as follows: nonsmokers or occasional smokers, non-alcoholics, occasional or no coffee drinkers, not drug users, without other
The patients received any treatment during the follow-up period. Levels (and during the follow-up period at regular time intervals. If who had breast cancer and fulfilled the inclusion criteria were included in the prospective study. Data on size, location, and time to metastases were determined in all of them by using available clinical and laboratory data (regular X-ray examinations, computer-assisted tomography (CAT) scans, isotope tracing and tumor markers CAE and CA 15-3) when the patient was first seen, and then rechecked regularly at two month intervals. Circulating concentration of prolactin was measured before therapy (baseline levels) and during the follow-up period at regular time intervals. If the patients received any treatment during the follow-up period (second line of treatment), prolactin was measured before and after the treatment. This group was further divided according to the concentration of circulating prolactin before treatment into two subgroups: hyperprolactinemic (n = 23), with an average circulating concentration of prolactin >520 mU/L, and normopro lactinemic (n = 23), with an average circulating concentration of prolactin between 52 mU/L and 520 mU/L. According to the presence of metastases, the patients were divided into two subgroups: patients with metastases (n = 39) and without metastases (n = 7).

There were two control groups of patients. The first control group consisted of 40 clinically healthy women with normal mammograms. The circulating concentrations of prolactin were measured in all of them at least three times during the observation period.

The second control group consisted of 33 female patients with cancer of different histological origin and location, mostly colorectal and lung cancer. This group was also divided according to the presence of metastases into two subgroups: patients with metastases (n = 23) and without metastases (n = 10).

All patients and controls signed informed consent forms before inclusion into the study. The follow up lasted 5 years.

**Metastatic Parameters**

The patients were in different stages of the disease and post-treatment periods. The patients were followed closely over the observation period and examined at weekly intervals. They underwent clinical and laboratory examinations and radiologic tests, if necessary, to check for possible metastases. The following metastatic parameters were determined: time interval to metastases, and location and size of metastatic deposits. In all patients, the exact date when they first palpated their lumps, the date when they first reported it to their physician, and the date of surgery were recorded. The majority of patients were surgically treated (79%), and those who were not (because of locally spread or infiltrated primary lesion, or the presence of supratacicular metastases) were treated conservatively, so the first day of treatment was recorded. The patients were followed-up at regular time intervals, or were treated and then regularly checked up for five years. Since they were followed-up very closely, the first occurrence of detectable metastatic deposits was accurately estimated. Time interval to metastases was calculated from the first day of surgery or other treatment.

The site and size of metastatic deposits were determined by CAT scans. The size of lung metastatic lesions, accompanied by atelectatic areas, was measured. The size of visceral metastases was estimated on the basis of CAT scans. If a patient had more metastatic deposits, their sizes were summed up and taken as a single metastasis, whose size was measured before and after the treatment. Metastatic deposits were classified according to their location and designated as follows: 1) local metastases, 2) regional lymph node metastases, 3) metastases in the opposite breast, 4) bone metastases, and 5) visceral metastases (liver, lungs, brain, or other organs).

**Blood Concentration of Prolactin**

Blood samples were drawn under sterile conditions at 8:00 a.m. and centrifuged at 3,000 rpm for 10 minutes at room temperature. Plasma was stored in plastic tubes at –20°C until processed. The circulating concentrations of prolactin were determined by means of radioimmunoassay (RIA) method with a commercial kit from Serono (Geneva, Switzerland). This method uses double antibody RIA principle and standard control – WHO/RP 75/504. Standard prolactin (1 ng of prolactin = 32.5 μg WHO 75/504), which has low cross-reaction with human gonadotropin hormones (0.15%) and low cross-reaction with thyroid stimulating hormone (0.39%). The range of normal values was 52-520 mU/L.

**Statistical Analysis**

The distribution of quantitative variables was determined by using distribution histograms with probability plots and de-trended probability plots. The results were evaluated with nonparametric Mann-Whitney test, with calculated mean values and standard deviations (SD), and Spearman’s correlation coefficient with two-sided significance of p-values. P < 0.05 was considered statistically significant. All statistical analyses were performed with SPSS statistical software, Version 10 (SPSS Inc, Chicago, IL, USA).

**Results**

**Circulating Concentrations of Prolactin**

Mean baseline prolactin concentrations (± standard deviation) were significantly higher in breast cancer patients than in healthy women (610 ± 442 mU/L vs 442 ± 107 mU/L, respectively; Mann-Whitney test, p = 0.04). They were also higher than in patients with other histological types of cancer at other sites (481 ± 273 mU/L; Mann-Whitney test, p = 0.02).

**Prolactin and Metastatic Parameters**

Metastases were not detected in only 7 out of 46 breast cancer patients during the 5-year follow-up period (Table 1). Five patients had local metastases, 4 had corresponding axillary lymph node metastases, 8 had bone metastases, and 22 had visceral metastases (Table 1). Metastases developed in all hyperprolactinemic patients, whereas about a third of normoprolactinemic patients were free of metastases.

The average time interval before the occurrence of metastases in 7 breast cancer patients with very high circulating prolactin concentrations before treatment was significantly shorter (U = 0; p < 0.001) than that in 14 patients with very low prolactin concentrations before treatment.

The average circulating concentration of prolactin in metastatic patients was significantly higher than that in non-metastatic patients. Such a difference was not found in comparison with patients with other histologic types and sites of cancer (Table 2).

The correlation between prolactin concentration and time to metastases was r = -0.3 (p = 0.03) in the

<table>
<thead>
<tr>
<th>Table 1. Site of metastases in relation to serum concentrations of prolactin in breast cancer patients</th>
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<tbody>
<tr>
<td>Patient group</td>
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<tr>
<td></td>
</tr>
<tr>
<td>All patients</td>
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<tr>
<td>Hyperprolactinemic</td>
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<tr>
<td>Normoprolactinemic</td>
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</table>
group of all breast cancer patients, and r = -0.5 (p = 0.03) in hyperprolactinemic patients (Table 3), indicating a slightly negative stochastic correlation between these two variables. Such a correlation was not detected either in normoprolactinemic (r = -0.3, p = 0.17) or in patients with other types and locations of cancer (r = -0.1, p = 0.52).

There was a positive correlation between circulating prolactin concentration before treatment and the size of metastases (r = 0.52, p = 0.001) in the group of all breast cancer patients and hyperprolactinemic breast cancer patients (r = 0.64, p = 0.001; Table 4). Such a correlation was not present in either the group of normoprolactinemic patients (r = 0.29, p = 0.18) or other cancer patients (r = 0.2, p = 0.26; Table 4). The patients who were hyperprolactinemic before treatment developed more massive metastases in shorter time.

The calculated correlation coefficient between prolactin before treatment and site of metastases in the group of breast cancer patients was r = 0.28 (p = 0.05; Table 5). There was a slightly positive stochastic correlation between these two variables, but calculated correlation coefficient was statistically insignificant. Such a correlation was not detected in either hyperprolactinemic (r = 0.33; p = 0.13) or normoprolactinemic patients (r = 0.33, p = 0.13) or in patients with other types and sites of cancer (r = 0.14, p = 0.43; Table 5).

Discussion

The average concentration of circulating prolactin before treatment was significantly higher in breast cancer patients than in healthy controls or patients with other histologic types and sites of cancer. This difference implied a specific association between prolactin and breast cancer, indicating a diagnostic and prognostic importance of prolactin concentration in this disease. However, previous measurements of serum prolactin in breast cancer patients have given controversial results. Some studies showed significant increase in circulating concentration of prolactin in breast cancer patients (8,13,14), and even in daughters of these patients (15), whereas other studies did not confirm these findings (12). The discrepancy between these results could at least in part be explained by the circadian rhythm in prolactin secretion and other factors possibly influencing its secretion (estrogen/progesterone balance, pregnancy and lactation, or various medicaments). In order for measurements to be reproducible and comparable, it is important to exclude all of these non-specific factors as much as possible.

In our study, metastases were detected in 39 out of 46 breast cancer patients during the 5-year follow-up period, and in the majority of cases they were visceral. The average concentration of circulating prolactin before therapy was significantly higher in metastatic patients than in those without metastases. Such a difference did not exist in the control group of patients with different types and sites of cancer. None of the patients without metastases had hyperprolactinemia, and all hyperprolactinemic ones had metastases. There also was a significant negative linear corre-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patient group</th>
<th>Prolactin (mU/L)</th>
<th>Size of metastases (cm²)</th>
<th>r*</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer (n = 46):</td>
<td>610 ± 442</td>
<td>24.1 ± 3.8</td>
<td>0.52</td>
<td>0.001</td>
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<tr>
<td>hyperprolactinemic breast cancer (n = 23)</td>
<td>929 ± 416</td>
<td>33.7 ± 6.5</td>
<td>0.64</td>
<td>0.001</td>
<td></td>
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<tr>
<td>normoprolactinemic breast cancer (n = 23)</td>
<td>292 ± 122</td>
<td>14.5 ± 2.9</td>
<td>0.29</td>
<td>0.18</td>
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<tr>
<td>Other cancer (n = 33)</td>
<td>524 ± 273</td>
<td>29.6 ± 11.2</td>
<td>0.20</td>
<td>0.26</td>
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</tbody>
</table>

*Spearman’s correlation coefficient.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patient group</th>
<th>Prolactin (mU/L)</th>
<th>Site of metastases (n* )</th>
<th>r*</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>With breast cancer (n = 46):</td>
<td>610 ± 442</td>
<td>3.4 ± 0.3</td>
<td>0.28</td>
<td>0.05</td>
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<tr>
<td>hyperprolactinemic breast cancer (n = 23)</td>
<td>929 ± 416</td>
<td>4.0 ± 0.3</td>
<td>0.33</td>
<td>0.12</td>
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<tr>
<td>normoprolactinemic breast cancer (n = 23)</td>
<td>292 ± 122</td>
<td>3.1 ± 0.2</td>
<td>0.33</td>
<td>0.13</td>
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<tr>
<td>Other cancer (n = 33)</td>
<td>524 ± 273</td>
<td>2.4 ± 0.4</td>
<td>0.14</td>
<td>0.43</td>
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</table>

*See Methods. Metastatic parameters.

*Spearman’s correlation coefficient.
luation between the concentration of circulating prolactin measured before the onset of treatment and time to metastases. A positive correlation was found with the size of metastases in a group of all breast cancer patients and hyperprolactinemic ones, as well as with cancer site in a group of breast cancer patients, whereas in patients with other sites of cancer such a correlation did not exist. These results suggested the association between the hyperprolactinemia and unfavorable prognostic factors of metastatic disease, such as shorter lag period between the occurrence of primary tumor and the appearance of metastases in hyperprolactinemic patients, larger size of metastases in hyperprolactinemic patients, and frequent presence of visceral metastases in hyperprolactinemic patients. It seems that breast cancer may be much more aggressive in hyperprolactinemic patients than in those with normal or below-normal concentrations of this hormone. The possibility of hyperprolactinemia being an indicator of progressive disease in breast cancer patients is supported by the results from other studies (8,13,16,17).

However, it still remains to be clarified whether hyperprolactinemia is the result or the cause of breast cancer. Further research on the relation between prolactin and disorders of the immune system (18-20) is needed to explain this problem. The results of a recent study (21) may provide new insights into how prolactin may stimulate disease progression. The new evidence is that prolactin seems to be a stimulator of the movement or motility of breast cancer cells, and it can actually trigger the invasive potential of those cells (21).

The origin of the circulating prolactin is also not clear. It is not yet known whether it originates exclusively from the pituitary gland, or perhaps from breast cells (both normal and cancerous). Our results showed a positive correlation between the size of metastatic tumor and the concentrations of circulating prolactin, favoring the opinion that endocrine prolactin plays greater role in the development and potentiation of the growth of breast cancer cells. The fact that antihyperprolactinemic drugs are very effective in lowering the circulating concentration of prolactin but ineffective in controlling the development of breast cancer in the majority of cases (22-24) supports the hypothesis that both endocrine and autocrine prolactin are involved in breast carcinogenesis. Probably the best explanation is that endocrine prolactin is responsible for initiation, and autocrine for perpetuation of carcinogenic process.

Acknowledgment
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